IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appln No.:	10/091,538	)	*Confirmation No. 8240*
Applicants:	Chatterjee et al.	)	
Filed:	March 7, 2002	)	
Title:	IMPROVED IN VITRO SYNTHESIS SYSTEM	)	This Appeal Brief was filed electronically on February 1, 2008 using the USPTO's EFS-Web.
Art Unit:	1652	)	
Examiner:	Rebecca E. Prouty	)	
Docket No.:	IVGN 300	)	

APPEAL BRIEF

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir or Madam:

This Appeal Brief is filed pursuant to the "Notice of Appeal to the Board of Patent Appeals and Interferences" filed on November 2, 2007. Applicants petition herewith for a one (1) month extension of time and provide the necessary fee for filing the Appeal Brief. Applicants hereby petition for a one (1) month extension of time. It is not believed that additional extensions of time or other fees are required beyond those that may accompany this electronic filing. However, if any further extension(s) of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned for under 37 C.F.R. § 1.136(a), and any fees required (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 50-3994.

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I. REAL PARTY IN INTEREST

Invitrogen Corporation is the assignee of the above-named patent application.

II. RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences known to appellant, the appellant's legal representative, or assignee that will directly affect, or be directly affected by or have a bearing on the Board's decision in the pending appeal.

III. STATUS OF CLAIMS

Claims 1, 16, 17, 28, 30, 41, 51-55, 57, 60-62, 69, 70, 77, 78, 85-87 and 91-96 presently stand twice and finally rejected by virtue of an office action mailed May 3, 2007. Claims 2-15, 18-27, 29, 31-40, 42-50, 56, 58, 59, 63-68, 71-76, 79-84, and 88-90 were canceled. Claims 1, 16, 17, 28, 30, 41, 51-55, 57, 60-62, 69, 70, 77, 78, 85-87 and 91-96 are the subject of this appeal.

IV. STATUS OF AMENDMENTS

The Amendment filed February 20, 2007 was entered and the Amendment under 37 C. F. R. §1.116 (After Final) filed December 14, 2007 has been entered. The Amendment under 37 C. F. R. §1.116 filed subsequent to the Final Office Action dated May 3, 2007 merely canceled withdrawn claims. The pending claims are shown in the Claims Appendix.

V. SUMMARY OF CLAIMED SUBJECT MATTER

There are 3 independent claims (Claims 1, 41 and 51) pending in this appeal. Claims 16-17, 28, 30, 55, 61-62 and 85-87 depend from independent claim 1. Claims 57, 69-70 and 91-93 depend from independent claim 41. Claims 52-54, 60, 77-78, and 94-96 depend from independent claim 51 .[1]

A. Independent Composition Claim 1

[1] None of the claims subject to the present appeal include any means-plus-function or step-plus-function claim recitations. Accordingly, as per 37 C.F.R. § 41.37(c)(1), there are no such recitations to be identified and mapped in a corresponding manner to the specification and drawings.

In claim 1, Applicants claim an in vitro protein or nucleic acid synthesis system. The system includes at least one extract from an E. coli cell that does not express Gam and that has reduced activity of a nuclease as a result of mutation. The extract is modified by the addition of Gam protein.

Independent claim 1 has been mapped below with the relevant supporting citations to the specification.

Claim 1	**Patent Specification**
An *in vitro* protein or nucleic acid synthesis system comprising:	Paragraph [0037]
at least one extract from an E. coli cell having a mutation that results in reduced activity of at least one nuclease,	Paragraph [0078] and [0100] Paragraph [0038] , [0039], [0048] and [0049]
wherein said E. coli cell does not express Gam,	Example 1, starting at paragraph [0106] and Example 8, starting at paragraph [0130]
wherein said at least one extract is modified by the addition of Gam protein. -	Paragraph [0038] and [0093]

B. Claims 16-17, 28, 30, 55 and 61-62 depend from claim 1

The *in vitro* synthesis system may further include at least one nucleic acid template selected from the group consisting of a DNA template and an RNA template (claim 16).[2] In one aspect, the *in vitro* syntheis system may include a DNA template and the *in vitro* system may be a transcription/translation system (claim 17).[3] The Gam protein utilized in the *in vitro* synthesis system may be a soluble Gam protein (claim 28).[4] The *in vitro* system may

[2] See paragraphs [0037], [0043], [0063] and [0073].
[3] See paragraph [0063]
[4] See paragraph [0115]

include at least one energy source (claim 30),[5] or at least two energy sources (claim 55).[6] The at least two energy sources may generate or regenerate high energy phosphate compounds (claim 85) [7]. The at least two different energy sources may include pyruvate, phosphoenolpyruvate (PEP), carbamoyl phosphate, acetyl phosphate, creatine phosphate, phosphopyruvate, glyceraldehyde-3-phosphate and glucose-6-phosphate (claim 86).[8] In one aspect, the at least two energy sources are phosphoenol pyruvate and acetyl phosphate (claim 87). [9]

The at least one nuclease in the in vitro system having reduced activity may be a DNase (claim 61).[10] The Dnase may include exonuclease I, exonuclease II, exonuclease III, exonuclease IVA, exonuclease IVB, RecBCD (exonuclease V), exonuclease VII, exonuclease VIII, RecJ, dRpase, endonuclease I, endonuclease III, endonuclease IV, endonuclease V, endonuclease VII, endonuclease VIII, endonuclease A, fpg, uvrABC, mutH, vsr endonuclease, ruvC, ecoK, ecoB, mcrBC, mcrA, mrr, topoisomerase I, topoisomerase II, topoisomerase III, or topoisomerase IV (claim 62)[11].

C. Independent Kit Claim 41

In claim 41, Applicants claim a kit for in vitro synthesis system. The kit includes at least one extract from an E. coli cell that does not express Gam and that has reduced activity of a nuclease as a result of mutation. The extract is modified by the addition of Gam protein. The kit may also include one or more nucleotides or derivatives thereof, one or more amino acids or derivatives thereof, one or more polymerases, one or more cofactors, one or more buffers or buffer salts, one or more energy sources, one or more nucleic acid templates, or one or more reagents to determine the efficiency of the kit or assay.

Independent claim 1 has been mapped below with the relevant supporting citations to the specification.

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[5] See paragraph [0037]
[6] See paragraph [0040]
[7] See paragraph [0075]
[8] See paragraph [0076]
[9] See paragraph [0090]
[10] See paragraph [0013]
[11] See paragraph [0048]

Claim 41	Patent Specification
A kit for *in vitro* synthesis comprising:	Paragraph [0037]
at least one extract from an E. coli cell having a mutation that results in reduced activity of at least one nuclease,	Paragraph [0078] and [0100] Paragraph [0038] , [0039], [0048] and [0049]
wherein said E. coli cell does not express Gam,	Example 1, starting at paragraph [0106] and Example 8, starting at paragraph [0130]
wherein said at least one extract is modified by the addition of Gam protein; and	Paragraph [0038] and [0093]
one or more nucleotides or derivatives thereof, one or more amino acids or derivatives thereof, one or more polymerases, one or more cofactors, one or more buffers or buffer salts, one or more energy sources, one or more nucleic acid templates, or one or more reagents to determine the efficiency of the kit or assay.	Paragraph [0045]

D. Claims 57, 69-70, 91-92 and 93 depend from claim 41

The kit for *in vitro* synthesis may include at least two energy sources (claim 57)[12] providing chemical energy for synthesis (claim 60).[13] Each of the at least two different energy sources generates or regenerates high energy triphosphate compounds for the synthesis (claim 91).[14] The at least two different energy sources may include pyruvate, phosphoenolpyruvate

[12] See paragraph [0041]
[13] See paragraph [0041]
[14] See paragraph [0075]

(PEP), carbamoyl phosphate, acetyl phosphate, creatine phosphate, phosphopyruvate, glyceraldehyde-3-phosphate and glucose-6-phosphate (claim 92).[15] In one aspect, the at least two energy sources are phosphoenol pyruvate and acetyl phosphate (claim 93). [16]

The at least one nuclease in the kit having reduced activity may be a DNase (claim 69).[17] The Dnase may include exonuclease I, exonuclease II, exonuclease III, exonuclease IVA, exonuclease IVB, RecBCD (exonuclease V), exonuclease VII, exonuclease VIII, RecJ, dRpase, endonuclease I, endonuclease III, endonuclease IV, endonuclease V, endonuclease VII, endonuclease VIII, endonuclease A, fpg, uvrABC, mutH, vsr endonuclease, ruvC, ecoK, ecoB, mcrBC, mcrA, mrr, topoisomerase I, topoisomerase II, topoisomerase III, or topoisomerase IV (claim 70)[18].

E. Independent Composition Claim 51

In claim 51, Applicants claim a composition that includes at least one extract from an E. coli cell that does not express Gam and that has reduced activity of a nuclease as a result of mutation. The extract is modified by the addition of Gam protein. The composition also includes at least one nucleic acid template in the presence of at least a partial syntheis product of the template.

Independent claim 1 has been mapped below with the relevant supporting citations to the specification.

Claim 51	Patent Specification
An *in vitro* protein or nucleic acid synthesis	Paragraph [0037]

[15] See paragraph [0076]
[16] See paragraph [0090]
[17] See paragraph [0013]
[18] See paragraph [0048]

Claim 51	Patent Specification
system comprising:	
at least one extract from an E. coli cell having a mutation that results in reduced activity of at least one nuclease,	Paragraph [0078] and [0100] Paragraph [0038] , [0039], [0048] and [0049]
wherein said E. coli cell does not express Gam,	Example 1, starting at paragraph [0106] and Example 8, starting at paragraph [0130]
wherein said at least one extract is modified by the addition of Gam protein, and	Paragraph [0038] and [0093]
at least one nucleic acid template in the presence of at least a partial synthesis product of said template.	Paragraph [0043]

F. Claims 52-54, 60 77-78 and 94-96 depend from claim 51

As claimed in claim 51, the composition includes at least one nucleic acid template in the presence of at least a partial synthesis product of the template. The product may be a nucleic acid product (claim 52) [19], and the nucleic acid product may be a DNA (claim 53) [20] or an RNA (claim 54) [21]. The claimed composition may further include at least two energy sources providing chemical energy for synthesis (claim 60).[22] Each of the at least two different energy sources generates or regenerates high energy triphosphate compounds for the synthesis (claim 94).[23] The at least two different energy sources may include pyruvate, phosphoenolpyruvate

[19] See paragraph [0063]
[20] See paragraph [0063]
[21] See paragraph [0063]
[22] See paragraph [0040]
[23] See paragraph [0075]

(PEP), carbamoyl phosphate, acetyl phosphate, creatine phosphate, phosphopyruvate, glyceraldehyde-3-phosphate and glucose-6-phosphate (claim 95).[24] In one aspect, the at least two energy sources are phosphoenol pyruvate and acetyl phosphate (claim 96). [25]

The at least one nuclease in the composition having reduced activity may be a DNase (claim 77).[26] The Dnase may include exonuclease I, exonuclease II, exonuclease III, exonuclease IVA, exonuclease IVB, RecBCD (exonuclease V), exonuclease VII, exonuclease VIII, RecJ, dRpase, endonuclease I, endonuclease III, endonuclease IV, endonuclease V, endonuclease VII, endonuclease VIII, endonuclease A, fpg, uvrABC, mutH, vsr endonuclease, ruvC, ecoK, ecoB, mcrBC, mcrA, mrr, topoisomerase I, topoisomerase II, topoisomerase III, or topoisomerase IV (claim 78)[27].

[24] See paragraph [0076]
[25] See paragraph [0090]
[26] See paragraph [0013]
[27] See paragraph [0048]

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Are claims 1, 16, 17, 28, 30, 41, 51-55, 57, 60, 85, 91 and 94 obvious over Pratt (*Coupled Transcription-Translation in Prokaryotic Cell-Free Systems*, Chapter 7 of Transcription and Translation: a Practical Approach Hanes, B.D., and Higgins, S.J., eds. (1984)) in view of Yu et al. (Proc. Natl. Acad. Sciences 97: 5978-5983 (2000))?

2. Are claims 86, 87, 92, 93 95 and 96 obvious over Pratt (*Coupled Transcription-Translation in Prokaryotic Cell-Free Systems*, Chapter 7 of Transcription and Translation: a Practical Approach Hanes, B.D., and Higgins, S.J., eds. (1984)) in view of Yu et al. (Proc. Natl. Acad. Sciences 97: 5978-5983 (2000)), and further in view of Swartz et al. (WO 00/55353)?

3. Are claims 61, 62, 69, 70, 77 and 78 obvious over Pratt (*Coupled Transcription-Translation in Prokaryotic Cell-Free Systems*, Chapter 7 of Transcription and Translation: a Practical Approach Hanes, B.D., and Higgins, S.J., eds. (1984)) in view of Yu et al. (Proc. Natl. Acad. Sciences 97: 5978-5983 (2000)), and further in view of Kudlicki et al. (U.S. Patent No. 6,664,379)?

VII. ARGUMENT

A. Claims 1, 16, 17, 28, 30, 41, 51-55, 57, 60, 85, 91 and 94 are not obvious over Pratt in view of Yu et al.

Of claims 1, 16, 17, 28, 30, 41, 51-55, 57, 60, 85, 91 and 94 and 20–22, claims 1, 41 and 51 are independent.

Independent Claims 1, 41 and 51 are Non-Obvious.

Claims 1, 41 and 51 recite an in vitro protein or nucleic acid synthesis system that comprises at least one extract from an E. coli cell that does not express Gam, in which the E. coli cell has a mutation that reduces the activity of at least one nuclease and in which the extract is modified by the addition of Gam protein. Applicants assert that i) a *prima facie* case for obviousness has not been made with respect to claims 1, 41 and 51; (ii) the cited references do not provide one of ordinary skill with any motivation to make an *in vitro* synthesis system with Gam protein, and (iii) the references do not provide one of ordinary skill with any reasonable expectation of success.

No Prima Facie Case of Obvious has been Established.

The MPEP states that to establish a *prima facie* case of obviousness there must be some suggestion or motivation in the prior art to make the claimed invention, there must be a reasonable expectation of success, and the prior art reference must teach or suggest all of the claim limitations. MPEP § 2142; In re Vaeck, 947 F.2d 488, 20 USPQ2d, 1438 (Fed. Cir. 1991). The reference teachings must be sufficient for one of ordinary skill in the relevant art having the reference before him or her to make the proposed substitution, combination, or other modification. In re Linter, 458 F.2d 1013, 1016, 173 USPQ 560, 562 (CCPA 1972). MPEP 2143.01. Further, in making a rejection based on obviousness, the Examiner must consider the invention as a whole. Bausch & Lomb v. Barnes-Hind/Hydrocurve, Inc., 796 F.2d 443, 447-49, 230 USPQ 416, 419-20 (Fed. Cir. 1986), cert. denied, 484 U.S. 823 (1987). MPEP 2141.02. Applicants assert that the requirements of a rejection under 35 U.S.C. §103(b) are not been met for at least the following reasons.

The cited references do not disclose each and every claim limitation. Independent claims 1, 41, and 51 recite an in vitro synthesis system, a kit, and a composition that include an E. coli cell extract, in which the E. coli cell used to make the extract has a mutation that reduces the activity of a nuclease, in which the cell does not express Gam, and in which the extract is modified by the addition of Gam protein. Neither Pratt nor Yu et al. disclose an extract and Gam protein as recited in amended claims 1, 41, and 51.

There is no Motivation Provided for Making an In Vitro Synthesis System With Added Gam Protein.

Neither Pratt or Yu et al., alone or in combination, suggest or provide motivation for making of an *in vitro* synthesis system with a cell extract that includes added Gam protein. Pratt, in discussing the use of linear DNA templates in E. coli *in vitro* synthesis systems, also does not anywhere suggest or provide motivation for the use of an inhibitor of recBC. Pratt in fact provides two alternative methods for preparing extracts having reduced reBC activity: the method of Zubay (pages 200-201) and the method of Gold and Schweiger (pages 201-202), both of which rely on the use of extracts of recB mutant cells and removal of DNA fragments from the extracts. Neither method includes the addition of a protein inhibitor of recB to a cell lysate used in the IVT system.

The Office Action of October 20, 2006 states that: "Yu et al. teach that one can alternatively inhibit the RecBCD exonuclease using the lambda phage Gam protein." Applicants do not agree that Yu et al. teach inhibition of a nuclease by addition of a protein, and particularly, as recited in the claims, by addition of a protein. Rather, Yu et al. teach *expression of the Gam gene within cells that also express RecBC nuclease* to allow genetic integration of linear DNA fragments to occur within the same cells. In asserting that Yu et al. provide motivation for the claimed invention, the Office Action further states on page 5 that ". . . the use of a protein inhibitor of recB as taught by Yu et al. would be substantially simpler than the method of Gold and Schweiger et al., requiring merely the addition of a protein to the ITT extract . . . a skilled artisan would clearly have been motivated to use the approach taught by Yu et al." Applicants dispute that Yu et al. teach the use of a protein inhibitor that requires "merely the addition of a protein" to the ITT extract. Yu et al. teach expression of a protein (Gam) within a live cell that is engineered to contain and express the Gam gene, and, further, the cell that is engineered by Yu et al. to express the Gam gene also produces the enzyme (recBC) that is to be inhibited by the in vivo synthesized protein (Gam). Thus, Yu et al. do not disclose an extract, do not teach addition of anything to any extract, and certainly do not teach the addition of a protein to an extract.

The References Provide No Reasonable Expectation of Success.

One of ordinary skill in the art, in adding Gam protein to an E. coli extract to produce an in vitro synthesis system, would not have a reasonable expectation of success in producing an in vitro synthesis system. At the time the invention was made, it was not known whether addition of Gam protein to an E. coli cell extract would protect linear DNA molecules from degradation, or if the use of Gam protein in an in vitro synthesis system would adequately reduce or eliminate recBC activity, or whether it would interfere with transcription and/or translation. Applicants have demonstrated in the specification, however, that the addition of Gam protein to an E. coli extract does protect a linear DNA from degradation (Example 6) and that addition of Gam protein to an in vitro synthesis system is not detrimental to protein synthesis from linear or supercoiled DNA templates, and leads to enhanced production of protein in systems having a linear DNA template (Example 7).

The present invention includes a cell extract modified by the addition of Gam protein, a feature of the invention not taught or suggested by the references, in which the protein can

reduce unwanted activity of an enzyme (recBC) at the appropriate time and in a titratable manner. Furthermore, no reasonable expectation of success was present at the time the invention was made, that in vitro protein or nucleic acid synthesis would occur in an extract that included added Gam protein.

Accordingly, Applicants therefore respectfully submit that the rejection under 35 U.S.C. §103(a) of claims 1, 41, and 51, and of claims 16, 17, 28, 30, 55, 61, 62, 85-87, and 94-96 that depend from claim 1, of claims 57, 69, 70, and 91-93 that depend from claim 41; and of claims 52-54, 60, 77, 78, and 94 that depend from claim 51, be removed and that these claims be viewed as allowable.

B. Claims 86, 87, 92, 93, 95 and 96 are not obvious over Pratt in view of Yu et al. and further in view of Swartz.

Claims 86 and 87 depend from claim 1, claims 92 and 93 depend from claim 41, and claims 95 and 96 depend from claim 51. For the reasons set forth above, Applicants assert that claims 1, 41, and 51 are nonobvious with respect to Pratt and Yu et al. Applicants assert that Swartz does not make up for the deficiencies of Pratt and Yu et al., in that Swartz also does not disclose or suggest Gam protein or the addition of Gam protein to an E. coli lysate. Thus, not all elements of claims 86, 87, 92, 93, 95, and 96 are presented in the cited references, and a prima facie case for obviousness under 35 U.S.C. §103(a) is not made. Claims 86, 87, 92, 93, 95 and 96 are therefore patentable under 35 U.S.C. §103(a) and Applicants respectfully request that the rejection be removed.

C. Claims 61, 62, 69, 70, 77 and 78 are not obvious over Pratt in view of Yu et al. and further in view of Swartz and further in view of Kudlicki et al..

Claims 61 and 62 depend from claim 1, claims 69 and 70 depend from claim 41, and claims 77 and 78 depend from claim 51. Applicants assert that no prima facie case for rejection under has been made, in that suggestion, motivation, and a reasonable expectation of success in adding Gam to an E. coli lysate are not present, and that in not all of the claims elements of the claimed in vitro synthesis system are present in Pratt and Yu, as provided in the arguments above. In particular, neither Pratt nor Yu et al. disclose Gam protein. Kudlicki does not make up

for the deficiencies of Pratt and Yu et al., in that Kudlicki does not disclose Gam protein. Thus, not all elements of claims 86, 87, 92, 93, 95, and 96 are presented in the cited references, and a prima facie case is not made. Claims 86, 87, 92, 93, 95 and 96 are therefore patentable under 35 U.S.C. §103(a) and Applicants therefore respectfully request that the rejection be removed.

Conclusion

In view of the foregoing, Applicants respectfully request reversal of the Examiner's final rejection.

Respectfully submitted,

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VIII. CLAIMS APPENDIX

1. An *in vitro* protein or nucleic acid synthesis system comprising:
at least one extract from an E. coli cell having a mutation that results in reduced activity of at least one nuclease, wherein said E. coli cell does not express Gam, wherein said at least one extract is modified by the addition of Gam protein. -

16. The *in vitro* synthesis system according to claim 1, further comprising at least one nucleic acid template selected from the group consisting of a DNA template and an RNA template.

17. The *in vitro* synthesis system according to claim 16, comprising at least one DNA template and wherein the *in vitro* synthesis system is an *in vitro* transcription/translation system.

28. The *in vitro* synthesis system according to claim 1, wherein said Gam protein is a soluble Gam protein.

30. The *in vitro* synthesis system according to claim 1, comprising at least one energy source.

41. A kit for *in vitro* synthesis comprising:

at least one extract from an E. coli cell having a mutation that results in reduced activity of at least one nuclease, wherein said E. coli cell does not express Gam, wherein said at least one extract is modified by the addition of Gam protein; and

one or more nucleotides or derivatives thereof, one or more amino acids or derivatives thereof, one or more polymerases, one or more cofactors, one or more buffers or buffer salts, one or more energy sources, one or more nucleic acid templates, or one or more reagents to determine the efficiency of the kit or assay.

51. A composition comprising:

at least one extract from an E. coli cell having a mutation that results in reduced activity of at least one nuclease, wherein said E. coli cell does not express Gam, wherein said at least one extract is modified by the addition of Gam protein, and

at least one nucleic acid template in the presence of at least a partial synthesis product of said template.

52. The composition according to claim 51, wherein the product is a nucleic acid product.

53. The composition according to claim 52, wherein the nucleic acid product is a DNA.

54. The composition according to claim 52, wherein the nucleic acid product is a RNA.

55 The *in vitro* synthesis system of claim 30, comprising at least two energy sources.

57. The kit of claim 41, comprising at least two energy sources.

60. The composition of claim 51, further comprising at least two energy sources providing chemical energy for synthesis.

61. The *in vitro* protein or nucleic acid synthesis system of claim 1, wherein said nuclease is a DNase.

62. The *in vitro* protein or nucleic acid synthesis system of claim 61, wherein said DNase is exonuclease I, exonuclease II, exonuclease III, exonuclease IVA, exonuclease IVB, RecBCD (exonuclease V), exonuclease VII, exonuclease VIII, RecJ,

dRpase, endonuclease I, endonuclease III, endonuclease IV, endonuclease V, endonuclease VII, endonuclease VIII, endonuclease A, fpg, uvrABC, mutH, vsr endonuclease, ruvC, ecoK, ecoB, mcrBC, mcrA, mrr, topoisomerase I, topoisomerase II, topoisomerase III, or topoisomerase IV.

69. The kit of claim 41, wherein said nuclease is a DNase.

70. The kit of claim 69, wherein said DNase is exonuclease I, exonuclease II, exonuclease III, exonuclease IVA, exonuclease IVB, RecBCD (exonuclease V), exonuclease VII, exonuclease VIII, RecJ, dRpase, endonuclease I, endonuclease III, endonuclease IV, endonuclease V, endonuclease VII, endonuclease VIII, endonuclease A, fpg, uvrABC, mutH, vsr endonuclease, ruvC, ecoK, ecoB, mcrBC, mcrA, mrr, topoisomerase I, topoisomerase II, topoisomerase III, or topoisomerase IV.

77. The composition of claim 51, wherein said nuclease is a DNase.

78. The composition of claim 77, wherein said DNase is exonuclease I, exonuclease II, exonuclease III, exonuclease IVA, exonuclease IVB, RecBCD (exonuclease V), exonuclease VII, exonuclease VIII, RecJ, dRpase, endonuclease I, endonuclease III, endonuclease IV, endonuclease V, endonuclease VII, endonuclease VIII, endonuclease A, fpg, uvrABC, mutH, vsr endonuclease, ruvC, ecoK, ecoB, mcrBC, mcrA, mrr, topoisomerase I, topoisomerase II, topoisomerase III, or topoisomerase IV.

85. The *in vitro* synthesis system according to claim 55, wherein each of the at least two different energy sources generates or regenerates high energy triphosphate compounds for the synthesis.

86. The *in vitro* synthesis system according to claim 85, wherein the at least two different energy sources are selected from the group consisting of pyruvate, phosphoenolpyruvate (PEP), carbamoyl phosphate, acetyl phosphate, creatine phosphate, phosphopyruvate, glyceraldehyde-3-phosphate and glucose-6-phosphate.

87. The *in vitro* synthesis system of claim 86, wherein two of the at least two energy sources are phosphoenol pyruvate and acetyl phosphate.

91. The kit of claim 57, wherein each of the at least two different energy sources generates or regenerates high energy triphosphate compounds for the synthesis.

92. The kit of claim 91, wherein the at least two different energy sources are selected from the group consisting of pyruvate, phosphoenolpyruvate (PEP), carbamoyl phosphate, acetyl phosphate, creatine phosphate, phosphopyruvate, glyceraldehyde-3-phosphate and glucose-6-phosphate.

93. The kit of claim 92, wherein two of said at least two energy sources are phosphoenol pyruvate and acetyl phosphate.

94. The composition of claim 60, wherein each of the at least two different energy sources generates or regenerates high energy triphosphate compounds for the synthesis.

95. The composition of claim 94, wherein the at least two different energy sources are selected from the group consisting of pyruvate, phosphoenolpyruvate (PEP), carbamoyl phosphate, acetyl phosphate, creatine phosphate, phosphopyruvate, glyceraldehyde-3-phosphate and glucose-6-phosphate.

96. The composition of claim 95, wherein two of said at least two energy sources are phosphoenol pyruvate and acetyl phosphate.

IX. EVIDENCE APPENDIX

NONE.

X. RELATED PROCEEDINGS APPENDIX

NONE.